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OCCURRENCE, PATHOGENICITY AND CHARACTERIZATION OF *Fusarium* sp. INCITING WILT IN TOMATO

S. Usha Rani* and N. Ramya,
Department of Pathology, Faculty of Agriculture, Annamalai University, Annamalai Nagar - - 608 002, Tamilnadu, India.

Abstract

In the present investigation, 14 isolates of Tomato wilt pathogen were obtained from major tomato growing areas of Thanjavur, Theni, Cuddalore, Dindigul and Madurai districts of Tamil Nadu. These isolates were recovered from infected roots during 2013 and 2014. Cultural, morphological and pathogenicity studies of these isolates revealed that majority of the isolates belonged to *Fusarium oxysporum f.* sp. *lycopersici* and only 2 to *F. solani*. In pathogenicity test, only *F. oxysporum f.* sp. *lycopersici* isolates caused the true wilt symptoms in tomato, while *F. solani* isolates caused root rots in seedlings. Thus, *F. oxysporum* was identified as the major cause of tomato wilt disease in Tamil Nadu and efforts will be made to find a suitable disease management method to contain this.

Key words: Tomato wilt, *Fusarium species*, Occurrence, Pathogenicity and Characterization.

1. Introduction

Tomato (*Lycopersicon esculentum* Mill.) is the most important tropical vegetable crop and is widely used throughout the world. It is a native of Andes region of South America. Tomato occupies second position amongst the vegetable crops in terms of production. The total production of tomato in the country in 1998 - 1999 was 8.27 MT from an area of 0.46 Mha. Tomato, as one of the vegetable crops and fruits, is very important in human nutrition. Fruit of tomato is rich in Vitamin A, B and C. The disease is one of the major limiting factors for stable production of tomato, since highly resistant cultivars have not been developed and effective control methods are not available. Tomato wilt causes plant mortality, reduction in its yield and ultimately economic loss to the growers. Fungal wilt diseases are caused by a group of soil borne fungi, with greatest number of wilt diseases being caused by species from the genera *Fusarium* and *Verticillium* (Green, 1981). These organisms infect their hosts by entering the vascular system, and are transported within the conductive xylem tissue (Green 1981). *Fusarium* species are ubiquitous, found in temperate, tropical, arctic and desert regions of the world (Nelson 1981). In tropical and subtropical region, tomato was ranked as highly praized commodity in vegetable marked. Theni, Dindigul and Madurai are main tomato growing areas of Tamilnadu and wilt is a major constraint in the successful cultivation of tomato crop in these regions. Hence, the present investigation was conducted to ascertain the cause of wilt disease in tomato plants.

****Corresponding author*: S. Usha Rani**
***E. mail*:** usharanirajasekar@gmail.com
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2. Materials and Methods

Isolation and identification of wilt pathogen.

Diseased plant samples showing typical wilt symptoms were collected from major tomato growing locations of Tamilnadu during 2013 and 2014 and the pathogens were isolated from the infected stem and root regions of the young and mature wilted cucumber plants. Small bits (2 -3 mm) from infected portion were surface sterilized with 0.1 % $HgCl_2$ solution for 30 sec and repeatedly washed with sterilized distilled water three times to remove the traces of $HgCl_2$ and then dried on sterilized blotter paper. The bits were aseptically transferred to petriplates containing Potato Dextrose Agar (PDA) and incubated at 25±2 °C. The cultures were purified by hyphal tip method (Hansen, 1926) and maintained on PDA slants in a refrigerator for further use. Pure culture of each of the isolated fungi thus obtained was subjected to further studies for their cultural and morphological characters. Observations were recorded regarding color and type of mycelium, sporulation, spore shape, size and color. The isolated fungi were identified on the basis of morphological observation and compared with standard manuals and texts (Owen, 1956; Booth, 1971). Growth rate per day on PDA of all the isolates was also studied.

Pathogenicity

The Pathogenicity test of the isolates was done by a root dip method (Namiki *et al*., 1994). Single spore isolates were grown in 500 ml Erlenmeyer flasks containing 250 ml PD broth at 25±2 °C for 5 days. The resulting culture was passed through double-layered muslin cloth. The filtrate was resuspended in distilled water and the conidia were adjusted to 1×10^7 conidial ml using haemocytometer. Tomato seedlings were grown in pot soil sterilized by autoclaving at 15 psi for 1 hour consecutively for 2 days. Tomato seedlings with fully expanded leaves were used for Pathogenicity test. The susceptible variety PKM-1 was used for Pathogenicity test. The healthy seedlings were removed from the pot, and their roots were washed gently with root system in the above conidial suspension of each transplanted in plastic pots (9 cm in dm). Three seedlings per pot were planted and four pots were used for the dipped in sterilized distilled water. External symptoms and vascular discolouration were observed after inoculation. After 28 days of inoculation the pathogens were reisolated from vascular bundles of inoculated plants. These were compared with the isolates used originally to inoculate the plants. Predominant pathogen which took minimum time to express the disease symptoms after inoculation was used for the further studies.

3. Results and Discussion

Fourteen isolates of pathogen were recovered from diseased plant samples collected from major cucumber growing areas of Thanjavur, Theni, Cuddalore, Dindigul and Madurai districts of Tamilnadu (Table - 1). On the basis of morphological and cultural characteristics (Table - 2), majority of the isolates (85.7 %) showed rapid growth rate (10.73 mm d^{-1}) on PDA medium. The mycelium was aerial and white in colour when young and turned to light purple or mauve at maturity and produced both micro and macroconidia. The microconidia were abundant with oblong to slightly reniform in shape, mostly non septate and rarely showing 1 - 2 septa with size 6.4 - 17.6 × 1.6 - 4.0 µm. The macroconidia were scarcely produced, cylindrical in the central portion, slightly curved and attenuated towards each end, somewhat pedicellate, mostly three septate and rarely four septate, of size 16.0 – 42 × 02.4 - 4.8 µm. The chlamydospores were abundant in older cultures which were in single or in pairs and were formed terminally or intercalary. The shape of the chlamydospores was globbose to ovoid, smooth and thick-walled measuring about 6.4 - 12.8 × 5.6 - 9.6 µm. These characters were compared with authentic literature (Owen, 1956; Booth, 1971) and designated as *Fusarium oxysporum f.* sp. *lycopersici*.

The second group of isolates comprised of only 14.3 % of the total isolates also showed rapid mycelium growth (10.50 mm d^{-1}) with dense and aerial mycelium which assumed pale to creamy shade at maturity. The microconidia were abundantly formed, oval in shape with single septa measuring 8.0 × 16.0 × 2.0 × 4.0 µm. In

comparison, the macroconidia were inequilaterally fusoid with well marked foot cell. The apical cell was pointed and slightly beaked. The size of macroconidia was 25.0 - 45.0 × 5.5 - 6.0 µm. The chlamydospores were abundantly formed, single or in pairs, terminal or intercalary with globose to oval in shape and measuring 9 - 12 × 9 - 10 µm. based on these characters the isolates were identified as *Fusarium solani* (Owen, 1956; Booth, 1971).

In pathogenicity test, the isolate of *F. oxysporum f.* sp. *lycopersici* proved to be virulent under artificial inoculation conditions on tomato cv. PKM-1 produced typical true wilt symptoms, resembling those incited by the fungus under natural conditions. Based on the disease symptoms after natural and artificial infection, the isolate was confirmed as *F. oxysporum f.* sp. *lycopersici*. On the contrary, *F. solani* did not produce typical true wilt but produced root rot of seedlings. On the basis of symptomatology, the pathogen was confirmed as *F. solani.*

The results (Table - 1) about the detail of pathogens associated with tomato wilt revealed presence of *F. oxysporum f.* sp. *lycopersici* and *F. solani*. The occurrence of *F. oxysporum f.* sp. *lycopersici* was more wide spread and was recovered from 12 out of 14 diseased samples. In contrast, *F. solani* was recovered from only 2 diseased samples. These results indicate predominance of *F. oxysporum f.* sp. *lycopersici* in causing wilt in tomato as the recovery of these isolates was made from diverse locations and constitute 85.7 % of the total isolates. The predominance of *F. oxysporum f.* sp. *lycopersici.* The causal agent of cucumber wilt has been reported by Hartman *et al*. (1991). In the present study, out of the two pathogens, only *F. oxysporum f.* sp. *lycopersici* caused true wilt in tomato plants during pathogenicity. These observations are also in line to the observations made by Jones *et al*. (1982) who while working on tomato wilt reported migration of mycelium of *F. oxysporum f.* sp. *lycopersici* rapidly into vascular bundles of tomato stems. Recovery of the pathogen *F. oxysporum f.* sp. *lycopersici*. Plants have also been reported by Padmadaya *et al*. (1996).

Table - 1: *Fusarium* species isolated from wilted tomato plants

Isolate No.	Identification	Growth stage affected	Location	District	Growth rate (mm d^{-1})
1.	*F. oxysporum f.* sp. *lycopersici*	Mature plant	TR-RI Aduthurai	Thanjavur	11.48
2.	*F. oxysporum f.* sp. *lycopersici*	Seedling	Thamarai kulam	Theni	10.31
3.	*F .oxysporum f.* sp. *lycopersici*	Mature plant	KVK Pallur	Cuddalore	9.34
4.	*F. oxysporum f.* sp. *lycopersici*	Mature plant	AC & RI Madurai	Madurai	11.07
5.	*F. oxysporum f.* sp. *lycopersici*	Mature plant	Chatranpatti	Dindigul	12.26
6.	*F. oxysporum f.* sp. *lycopersici*	Seedling/Mature plant	Papanasam	Thanjavur	9.48
7.	*F. oxysporum f.* sp. *lycopersici*	Seedling	HC & RI Theni	Theni	10.14
8.	*F. oxysporum f.* sp. *lycopersici*	Seedling/Mature plant	Annamalai University	Cuddalore	11.53
9.	*F. oxysporum f.* sp. *lycopersici*	Mature plant	Ottanchathiram	Dindigul	10.26
10.	*F. oxysporum f.* sp. *lycopersici*	Seedling	Mannargudi	Thanjavur	11.14
11.	*F. oxysporum f.* sp. *lycopersici*	Mature plant	kambam	Theni	11.83
12.	*F. oxysporum f.* sp. *lycopersici*	Seedling/Mature plant	Mellur	Madurai	9.93
13.	*F. solani*	Seedling/Mature plant	Sivapuri	Cuddalore	10.31
14.	*F. solani*	Mature plant	Salligramam	Thanjavur	10.68

Table - 2: Morphological and cultural features of *Fusarium species* isolated from wilted tomato during 2013-2014

***Fusarium* species**	**Mycelial colour and growth**	**Conidia**		**Chlamydospores**	**Average growth rate (mm d^{-1})**
		Microconidia	**Macroconidia**		
F. oxysporum f. sp. *lycopersici* (12 isolates)	Rapid growth, white aerial mycelium in young cultures and assumed shades of purple (mauve to auricular purple) at maturity.	Abundant, oblong to slightly reniform, non - septate, rarely 1 or 2 septate, 6.4 – 17.6 × 1.6 – 4.0 µm.	Scares, nearly cylindrical in central portion, slightly curved and attenuated towards each end, somewhat pedicellate, mostly three septate, four septate rare 16.0 - 42.0 × 2.4 – 4.8 µm.	Abundant in older cultures, single or in pairs, terminal or intercalary, globose or ovoid, smooth and thick - walled measuring 6.4 – 12.8 × 5.6 -9.6 µm.	10.73
F. solani (2 isolates)	Rapid growth, dense and floccose, aerial mycelium assumed pale to cream shade.	Abundant, microconidia are broader and more oval in shape with somewhat thicker walls, 1 – septate, 8.0 – 16.0 × 2.0 - 4.0 µm.	Inequilaterally fusoid, many cells had a rounded foot cell but few cells had well – marked foot cell. The apical cell has pointed and somewhat beaked, 25.0 – 45.0 × 5.5 – 6.0 µm.	Abundant , single or in pairs, terminal or intercalary globose to oval, smooth – walled, 9 – 12 × 8 - 10 µm.	10.50

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